



ab119691

GFP Dipstick ELISA Kit

Instructions for Use

For rapid determination of GFP protein expression

This product is for research use only and is not intended for diagnostic use.

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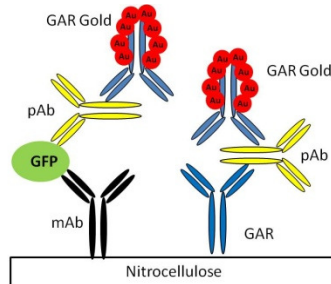
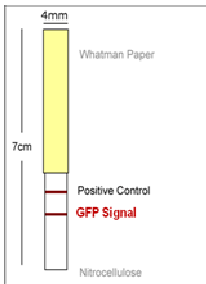
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1. Introduction

Principle: ab119691 GFP Dipstick ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro lateral flow immunoassay for the rapid determination of GFP or GFP tagged protein expression in cell and tissue lysates. The assay employs three antibodies: (1) a mouse monoclonal antibody (mAb) specific to GFP immobilized on a nitrocellulose membrane in a thin line perpendicular to the length of the dipstick, (2) a rabbit polyclonal (pAb) antibody specific to GFP and (3) a gold-conjugated goat anti-rabbit (GAR) antibody with very low cross-reactivity against mouse antibodies. Standards and samples are wicked past the mAb immobilized on the dipstick, followed by sequential wicking of the pAb and the gold-conjugated GAR in a similar fashion. When GFP is present in the sample, a red line appears at the site of the anti-GFP antibody line. The signal intensity is directly related to the amount of GFP in the sample. The signal intensity is best measured by a dipstick reader or may be analyzed by another imaging system or by simple visual inspection in the presence of a calibration curve.

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Background: Green fluorescence protein (GFP) is a 27 kDa protein derived from the jellyfish *Aequorea victoria*, which emits green light (emission peak at a wavelength of 509 nm) when excited by blue light (excitation peak at a wavelength of 395 nm). GFP has become an invaluable tool in cell biology research, since its intrinsic fluorescence can be visualized in living cells. GFP has been widely used as a reporter for gene expression, enabling researchers to visualize and localize GFP-tagged proteins within living cells without the need for chemical staining.

While GFP expression can sometimes be detected by fluorescence emission in cells and tissues, determination of very low levels of expression for optimization of expression is difficult and time consuming. This GFP Dipstick ELISA Kit is lateral flow immunoassay developed for an easy, rapid and sensitive detection of GFP or GFP fusion proteins in cells or tissue samples. GFP may be accurately quantified with the use of an immunochromato-reader by comparing

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the signal from an unknown sample with that of a known recombinant GFP standard curve. The kit has a detection sensitivity limit of 50 pg/mL GFP. Each kit provides sufficient reagents to perform up to 48 assays including standard curve and GFP samples. Note, it is possible that improper folding of GFP or the presence of a fusion partner may prevent GFP binding in this kit.

2. Assay Summary

Bring all reagents, samples and dipsticks to room temperature.

Prepare all the reagents, samples, and standards as instructed.



Wick 25 μL of standard or sample on the dipstick (~5 min)



Wick 25 μL of diluted detector antibody on the dipstick (~10 min).



Reconstitute gold conjugated GAR and wick on the dipstick
(~10 min)



Wash dipstick by wicking 50 μL of the wash buffer (~15 min).



Air dry dipsticks (~ 10 min)



Measure signal or visualize against a calibration curve

3. Kit Contents

Dipsticks	48
GFP Microplate (2 x 24 Wells)	48 Wells
10X Detector Antibody	0.14 mL
Extraction Buffer	25 mL
10X Blocking Buffer	1 mL
Wash Buffer	5 mL
GFP Protein Standard (100 ng)	1 vial

4. Storage and Handling

Store all components at 4°C. This kit is stable for 6 months from receipt. After reconstitution the standard should be stored at -80°C. 10X blocking, dipsticks and gold-conjugated GAR may be stored at room temperature in the provided containers and out of direct sunlight.

5. Additional Materials Required

- Method for determining protein concentration (BCA assay recommended)
- Pipetting devices
- Optional: Protease inhibitors
- Optional: Dipstick reader or other imaging system (i.e. flat bed scanner or blot imaging station)
- Optional: 37°C incubator/oven for fast drying of the dipstick

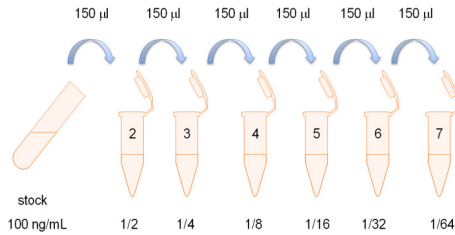
6. Preparation of Reagents

1. Bring all reagents and dipsticks to room temperature (18-25°C) before use.
2. Prepare 1X Antibody Dilution Buffer by adding 300 μ L of 10X Blocking Buffer + 300 μ L of Extraction Buffer + 2.4 mL of Wash Buffer. After performing the dipstick assay unused 1X Antibody Dilution Buffer should be stored at -20°C for 6 months.
3. Prepare 1X Sample Dilution Buffer by adding 500 μ L of 10X blocking buffer + 4.5 mL of Extraction Buffer. After performing the dipstick assay unused 1X Sample Dilution Buffer should be stored at -20°C for 6 months.

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4. Dilute the 10X Detector Antibody 10-fold with 1X Antibody Dilution Buffer immediately before use. Prepare 30 μL per dipstick.
5. Reconstitute the GFP Protein Standard (100 ng) with 1 mL of 1X Sample Dilution buffer by pipetting. Allow to sit for 10 minutes and repeat pipetting to ensure thorough reconstitution. This 100 ng/mL stock of standard material is then used to generate a standard curve in labeled tubes as described below. Any remaining stock material can be stored at -80°C for 6 months.
6. Label tubes #2-7. Label the 100 ng/mL GFP Protein Standard stock tube #1. Add 150 μL of 1X Incubation buffer to each of #2 through #7. Transfer 150 μL from tube #1 to tube #2. Mix thoroughly. With a fresh pipette tip transfer 150 μL from #2 to #3. Mix thoroughly. Repeat for Tubes #4 through #7. Use 1X Sample Dilution buffer as the zero standard tube labeled #8. For accurate quantification of GFP expression, use a freshly prepared serially diluted standards for each assay. For qualitative assessment, use 1 standard per kit lot.

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7. Sample Preparation

Note: Extraction buffer can be supplemented with phosphatase inhibitors, PMSF and protease inhibitor cocktail prior to use. Supplements should be used according to manufacturer's instructions.

7.1 Cell lysates.

- 7.1.1. Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 10 min at 4°C.
- 7.1.2. Rinse cells twice with PBS.
- 7.1.3. Solubilize cell pellet at 2×10^7 /mL in Extraction Buffer. Enough extraction buffer is provided for 40 samples, each extracted in 500 µL volume.
- 7.1.4. Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay

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samples immediately or aliquot and store at -80°C for 6 months. The sample protein concentration in the extract may be quantified using a protein assay.

Note: Do not determine protein concentration by the A280 method.

7.2 Tissue lysates.

7.2.1. Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

7.2.2. Suspend the homogenate to 25 mg/mL in PBS.

7.2.3. Solubilize 100 μL of the homogenate by adding 4 volumes of Extraction Buffer to a sample protein concentration of 5 mg/mL.

7.2.4. Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C . Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C for 6 months. The sample protein concentration in the extract may be quantified using a protein assay.
Note: Do not determine protein concentration by the A280 method.

7.3 Sub-cellular organelle lysates e.g. mitochondria.

- 7.3.1. Prepare the organelle sample by, for example, sub-cellular fractionation.
- 7.3.2. Pellet the sample.
- 7.3.3. Solubilize the pellet by adding 9 volumes of Extraction Buffer.
- 7.3.4. Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C for 6 months. The sample protein concentration in the extract may be quantified using a protein assay.

8. Assay Procedure

Equilibrate all reagents, samples and dipsticks to room temperature before use. It is recommended all samples and standards be assayed in duplicate. Wicking of volume onto the dipstick should be done until the entire volume has been absorbed. Do not allow the dipsticks to dry before the wash step has been completed. Do not handle dipsticks without gloves.

- 8.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

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- 8.2. Remove number of desired dipsticks from the container and label each dipstick on the Whatman paper (heavy end) with the sample name. Keep excess dipsticks in the provided closed container and return to storage.
- 8.3. Add 25 μL of each diluted standard or sample per well on an empty well of the provided gold-conjugated antibody plate (i.e. row A or E). It is recommended to include a dilution series of GFP protein as a reference. Also include a 1X Sample Dilution Buffer as a zero standard.
- 8.4. Add 25 μL of the diluted detector antibody on an empty well of the provided gold-conjugated antibody plate (i.e. row B or F).
- 8.5. Reconstitute the gold-conjugated antibody (present in rows C and G) by adding 25 μL /well of Antibody Dilution buffer.
- 8.6. Add 50 μL of Wash buffer on an empty well of the provided gold-conjugated antibody plate (i.e row D or H).
- 8.7. Gently add a dipstick to the well with sample (i.e A1) by placing the thin/nitrocellulose end of the dipstick down. The dipstick must reach the bottom of the well.
- 8.8. Allow the entire sample volume in the well to wick up onto the dipstick.

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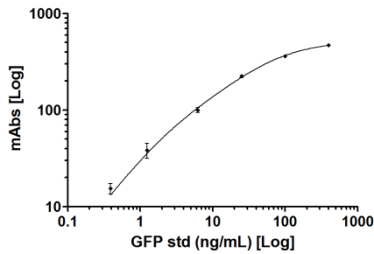
- 8.9. Transfer the dipstick to the well containing the detector antibody (i.e B1). Allow the entire volume in the well to wick up onto the dipstick.
- 8.10. Transfer the dipstick to the well containing the reconstituted gold-conjugated antibody (i.e. C1). Allow the entire volume in the well to wick up onto the dipstick. The control GAR band should appear in a approximately 2-5 minutes.
- 8.11. Transfer the dipstick to the well containing the wash buffer. Allow to the entire volume in the well to wick up onto the dipstick (i.e D1).
- 8.12. Air dry dipsticks at room temperature or 37°C.
- 8.13. Read dipsticks.

9. Data Analysis

9.1 INSTRUMENT-BASED (IMMUNOCHROMATO-READER)

Insert the dipstick (nitrocellulose first) into the housing located inside the reader's holder tray. The immunochromato reader will give an absorbance intensity number based on the intensity of the colored line. Average the duplicate standard readings and plot against their concentrations after subtracting the zero standard reading. Draw the best smooth curve through these points to construct a standard curve. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Read GFP protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in 1X Sample dilution buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

TYPICAL STANDARD CURVE - For demonstration only.



Standard (ng/mL)	mAbs
0.39	13.5
1.25	45.0
6.25	106.3
25	232.8
100	358.3
400	484.5

Figure 1. Example standard curves.

TYPICAL SAMPLE RANGE

Sample	Range
GFP (pure protein)	0.050 - 400 ng/mL

SENSITIVITY

Calculated minimum detectable dose = 50 pg/mL (zero dose n=8 +2 standard deviations).

LINEARITY OF DILUTION

1 mg/mL Hela spiked with GFP (ng/mL)	% Expected
400	99%
100	80%
25	92%
6.25	112%
1.25	90%
0.39	86%

REPRODUCIBILITY

Parameter	%CV
Intra (n= 6)	7.5
Inter (n=3 days)	8.3

RECOVERY

Sample Type	Average Recovery %	Range %
Cell Culture Media	164	133 - 200

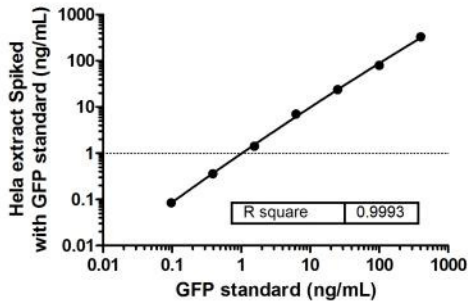


Figure 2. Example of the recovery with GFP standard spiked into diluted HeLa cell extract.

9.2 INSTRUMENT-FREE, VISUAL READOUT

The calibration curve generated with the standard protein can be made into a “visual reference card”. The test sample is then compared against the visual reference card by matching up the reporter line density of the processed dipstick in the appropriate slot (See Figure 2). The value (or value range) can then be interpolated based on the position relative to the antigen levels of the two lines immediately to the left and to the right. The accuracy of the value will be inversely proportional to the dilution factor of the calibration curve.

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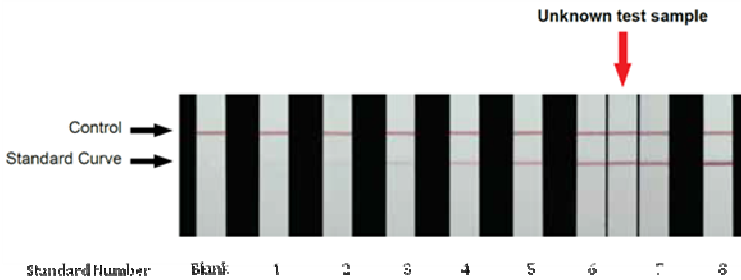


Figure 3. Dipstick Visual Reference Card Interpolation.

The unknown test sample reporter line fits neatly into the slot between the standard #6 and 7.

Accuracy of visual inspection was tested by running a calibration curve with the standard GFP in a 1:4 dilution from 100ng/mL to 0.4ng/mL. Twelve naïve testers were shown three test dipsticks (not part of the calibration curve) which were previously analyzed with the immunochromaturo-reader and interpolated from the curve obtained. By visual inspection, the GFP signal showed a percentage error of 15% in the 100 – 25ng/mL range, 6% in the range of 25 – 6.25ng/mL range and 10% in the range of 0.3 – 1.5ng/mL range (n = 12) from the exact reading obtained with the immunochromaturo reader. The same dipsticks shown to naïve testers were scanned on a flat bed scanner and analyzed with Image J software. Percentage error was calculated from the gold standard reading on the immunochromaturo-reader and was found 54% in the 100 – 25ng/mL range,

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22% in the 6.25 – 25ng/mL range and 9% for the 1.5 – 6.25ng/mL range.

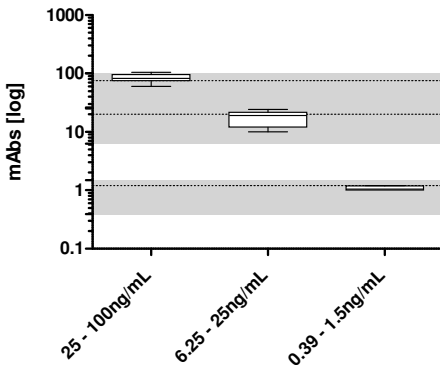
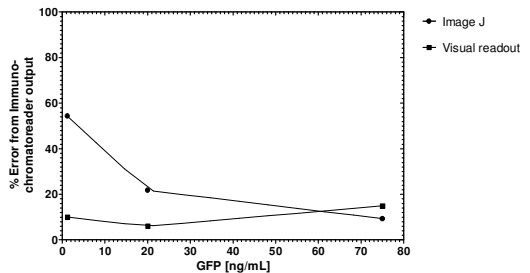


Figure 4. Accuracy of visual inspection. The X axis shows the range between the dipsticks of the calibration curve (graphed as gray boxes against the Y axis). The Y axis shows the median estimated value by 12 naïve testers. The error shows the 25 – 75 percentiles from the median.

Figure 5. Comparison between visual inspection and imaging analysis method. % Error for each point is shown on the Y axis.



10. Specificity

This kit is reactive with *Aequorea victoria* GFP but is untested and may not react with other fluorescent protein variants (RFP, YFP, CFP, etc). Also note it is possible that improper folding of GFP or the presence of a fusion partner may prevent GFP binding in this kit.

11. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly spin the vial of Item C prior to opening and dissolve the powder thoroughly by a gentle mix.
Poor standard curve	Dipsticks were cold and moist during the assay	Ensure that dipsticks warm up to room temperature before use. Store them at room temperature
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Sample, detector or gold were not fully wicked	Ensure that wells are empty before moving dipstick to the next step
	Sample is too viscous and will not wick from dipstick to dipstick	Dilute the sample
Low sensitivity	Improper storage of the kit	Store your standard at -80°C after reconstitution, Keep Wash Buffer and Extraction Buffer at 4°C. Keep dipsticks, gold and block at RT. Keep 1X Detectors and 1X Sample Dilution Buffer at -20C.
No signal on GAR control dipstick line	Detector antibody was not added	Ensure detector antibody is added during the assay procedure.

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Abcam in the USA

Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)

Fax: 866-739-9884

Abcam in Japan

Abcam KK
2-2-1 Nihonbashi
Horidome-cho,
Chuo-ku, Tokyo
103-0012
Japan

Tel: +81-(0)3-6231-094

Fax: +81-(0)3-6231-0941

Abcam in Europe

Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000

Fax: +44 (0)1223 771600

Abcam in Hong Kong

Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682

Fax: (852) 3016-1888